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## The Effects of Zinc and Vitamin C Supplementation on the Glycemic Profile in Type 2 Diabetic Patients

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### Abstract

Diabetes mellitus (DM) is the most common endocrine disorder and one of the highly progressive diseases which is indicated by high blood sugar (hyperglycemia). The present study aims to study the effects of treatment with zinc and vitamin C on glycemic profile [fasting blood sugar (FBS), glycated haemoglobin (HbA<sub>1c</sub>), and insulin hormone level] in type 2 diabetic patients. The study included 56 diabetic patients [30 without foot ulcers (15 without zinc and vitamin C supplements, 15 with zinc and vitamin C supplements) and 26 with foot ulcers (15 without zinc and vitamin C supplements, 11 with zinc and vitamin C supplements)]. Also, 15 healthy persons were used as a control group. The patients were enrolling to Azadi Teaching Hospital and Kirkuk General Hospital/ Kirkuk city/ Iraq; their ages ranged from 30 to 60 years and weights ranged from 70 to 90 kilogram. Blood samples were collected from the studied subjects to determine fasting blood sugar (FBS) concentration, glycated haemoglobin (HbA<sub>1c</sub>) level, and insulin hormone level. Zinc and vitamin C supplements were used for four weeks by a group of the patients. The results showed highly significant ( $P \leq 0.01$ ) decreases in the values of FBS, HbA<sub>1c</sub>, and insulin in diabetic patients treated with supplements (both with and without foot ulcers) compared with diabetic patients without supplements (both with and without foot ulcers). It can be concluded that zinc and vitamin C supplements have improving impacts on patients with diabetes mellitus by improving glycemic profile parameters, such as FBS, HbA<sub>1c</sub>, and insulin.

**Keywords:** T2DM, glycemic profile, zinc, vitamin C.

دراسة تأثير الزنك وفيتامين C على المؤشرات المرتبطة بسكر الدم لدى مرضى السكري من النوع

الثاني

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الخلاصة

مرض السكري هو المرض الأكثر شيوعاً ضمن امراض اضطرابات الغدد الصماء، وواحد من أكثر الأمراض شديدة التقدم. والذي يتميز بارتفاع نسبة السكر في الدم. تهدف الدراسة الحالية إلى دراسة تأثيرات الزنك وفيتامين C على المؤشرات المرتبطة بسكر الدم [سكر الدم الصائم، الهيموغلوبين السكري، ومستوى هرمون الأنسولين] في مرضى السكري من النوع الثاني. في هذه الدراسة، ستة وخمسون مريضاً مصاباً بداء السكري [30 مريض بدون قرح القدم (15 بدون اعطاء مكملات الزنك وفيتامين C، و 15 مع اعطاء مكملات الزنك وفيتامين C) و 26 مصاباً بقرح القدم (15 بدون اعطاء مكملات الزنك وفيتامين C، و 11 مع اعطاء

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مكملات الزنك وفيتامين C] تطوعوا، كما شارك في هذه الدراسة خمسة عشر شخصاً سليماً كمجموعة سيطرة من مستشفى آزادي التعليمي ومستشفى كركوك العام/ مدينة كركوك/ العراق. تراوحت أعمارهم بين (30 الى 60 سنة)، وتراوحت أوزانهم بين 70 الى 90 كيلوغراماً. تم جمع عينات الدم من الخاضعين للدراسة لتحديد تركيز السكر في الدم الصائم ومستوى الهيموجلوبين السكري ومستوى هرمون الأنسولين. تم استخدام مكملات الزنك وفيتامين C لمدة أربعة أسابيع من قبل المرضى. أظهرت النتائج أن مستويات سكر الدم الصائم والهيموجلوبين السكري وهرمون الأنسولين انخفضت معنوياً ( $P \leq 0.01$ ) في مرضى السكري الذين يتناولون المكملات (سواء مع أو بدون قرح القدم) مقارنة بمرضى السكري بدون مكملات (سواء مع أو بدون قرح القدم). يمكن الاستنتاج من نتائج الدراسة الحالية أن مكملات الزنك وفيتامين C لها تأثيرات معززة على داء السكري عن طريق تحسين المؤشرات المرتبطة بسكر الدم مثل سكر الدم الصائم، الهيموجلوبين السكري، وهرمون الأنسولين.

## Introduction

Diabetes mellitus (DM) is the most common endocrine disorder and one of the highly progressive diseases that is characterised by high blood sugar levels (hyperglycemia) [1, 2]. It is considered as one of the fastest growing non-communicable health difficulties in the current century worldwide [3]. Diabetes mellitus is categorized into many types, including type 1 diabetes mellitus; type 2 diabetes mellitus, gestational diabetes mellitus, and others [4]. Type 2 diabetes mellitus (T2DM) is considered as the most expressive type of this disease [5], constituting approximately 85-95% of the whole DM cases [6]. Type 2 diabetes mellitus is characterized by hyperinsulinaemia, insulin resistance (may range from predominant insulin resistance with proportional insulin deficiency to a dominant secretion defect with insulin resistance), and  $\beta$ -cell dysfunction [7, 8]. It is also named non-insulin dependent diabetes mellitus (NIDDM) [4]. It happens when the pancreas does not produce sufficient insulin or when the body does not efficiently use the produced insulin [8].

Under normal conditions, blood glucose homeostasis is quite well-maintained by organized control of several hormones, such as insulin and glucagon, in addition to cytokines. However, chronic exposure to a diabetic environment with high sugar / high fat diets and physical/ mental stress can cause hyperglycemia, which is one of the main characteristics of insulin hormone resistance, metabolic syndrome, and diabetes mellitus [9].

Insulin is the principal glucose regulatory hormone [10]. It is produced by beta ( $\beta$ ) cells of the endocrine pancreas within specialized areas called the islets of Langerhans [11]. Primarily, insulin increases the facilitated transport of glucose into the cells, thus reducing blood glucose level [10]. It is stored as a hexamer, containing two zinc ions, in the pancreatic  $\beta$ -cells inside secretory granules or vesicles, where the two  $Zn^{++}$  ions organize six insulin monomers to create the hexameric-structure on which the matured insulin crystals are based [7, 12]. In the disease state, the resistance to insulin in the skeletal muscles, added to the increase in gluconeogenesis in the liver, lead to elevated blood glucose levels, which compels beta cells to produce more insulin. This leads to the exhaustion of beta cells and, upon prolonged hyperglycemia, their apoptosis, causing progression of the disease [13]. Accordingly, the secretion of insulin is strictly controlled by glucose, other hormones, and the autonomic nervous system [14].

Glycated haemoglobin is an indirect measure of average blood glucose levels [15]. Diagnosis of diabetes is based on the threshold of  $HbA_{1C}$ , which is an indicator of developing microvascular complications.  $HbA_{1C}$  is also a continual cardiovascular (CV) risk factor and a better predictor for CV events than fasting blood glucose (FBG) or 2 hour postprandial glucose (2h PPG). Several advantages of using  $HbA_{1C}$  in diabetes diagnosis were reported, which include the possibility of measurement at any times of the day and the higher convenience of testing as compared to FBG or 2hPG. Also,  $HbA_{1C}$  test avoids the problem of

day-to-day variability of glucose level, as it reflects the average plasma glucose (PG) during the previous 2 to 3 months [8]. Several factors that may independently influence hemoglobin glycation should be considered, including age, anemia/hemoglobinopathy, and race/ethnicity [15].

Zinc (Zn) is vital nutrient for human health [16]. It is a micronutrient with a well-known antioxidant activity [17]. It is essential for the growth and development of animals, plants, and microorganisms. It has critical effects in homeostasis, oxidative stress, immune function, aging, and apoptosis. Significant disorders of high public health interests are related with zinc deficiency [18]. Vitamin C is one of nine water-soluble vitamins that is considered as a powerful dietary antioxidant. It is a generous donor of electrons; therefore, it is aiding in the scavenging mechanism [10]. Vitamin C is known as ascorbic acid (AA), which is the reduced form of Vitamin C [19]. It is essential for the normal physiological functions of the body [20]. The present study aims to study the effects supplementation of zinc and vitamin C on glycemic profile in type 2 diabetic patients.

### **Materials and methods**

This study was conducted according to the guidelines described in the Declaration of Helsinki. All procedures involving human patients were approved by the ethics committee of University of Baghdad (Approval number 3006 in 9/10/2018) and the ethics committee of Kirkuk Health Department (29877 in 15/10/2018). Verbal informed consent was obtained from all the patients, witnessed, and formally recorded.

The studied subjects were enrolling to Azadi Teaching Hospital and Kirkuk General Hospital/ Kirkuk city, Iraq. Seventy-one participants were distributed in five groups; the first group (G: A) included 15 apparently healthy non-diabetic subjects (7 males and 8 females) as a control group. The second group (G: B) involved 15 diabetic patients without foot ulcers and without supplements (6 males and 9 females). The third group (G: C) consisted of 15 diabetic patients with foot ulcers and without supplements (6 males and 9 females). The fourth group (G: D) included 15 diabetic patients without foot ulcers and with supplements (5 males and 10 females). The fifth group (G: E) included 11 diabetic patients with foot ulcers and with supplements (7 males and 4 females). The ages of the studied subjects ranged from 30 to 60 years, with the mean age values in the studied groups were as follows: G: A ( $47.40 \pm 1.84$  years), G: B ( $50.40 \pm 1.90$  years), G: C ( $52.20 \pm 1.61$  years), G: D ( $49.40 \pm 1.98$  years), G: E ( $51.63 \pm 2.49$  years).

The duration of the disease ranged from 5 to 23 years. The mean duration values in the studied groups were as follows: G: B ( $7.13 \pm 0.66$  years), G: C ( $14.00 \pm 1.49$  years), G: D ( $8.00 \pm 0.96$  years), G: E ( $14.90 \pm 1.93$  years). The weight range was from 70 to 90 kilogram. Obese and/or alcoholic patients were excluded from the study.

The patients were previously diagnosed with diabetes mellitus according to the hospitals' protocol. Based on this study design, the patients received 50 mg zinc oxide tablets at the morning and 500 mg vitamin C tablets after lunch every day, for four weeks.

Venous blood samples (5 ml) were collected at the morning from the studied subjects who were in a fasting state for 8 hours. 3 ml of the blood was transferred to a clean dry gel tube for the tests of serum FBS and insulin level and allowed to clot at  $37^{\circ}\text{C}$  for 30 minutes. The tube was then centrifuged at 5000 rpm for 5 minutes and serum was collected; 10  $\mu\text{L}$  was immediately used for FBS test and the rest was kept at  $-20^{\circ}\text{C}$  until used for serum insulin level test. The other part of the blood (2 ml) was directly transferred to Ethylenediaminetetraacetic acid (EDTA) -coated tube for glyated hemoglobin ( $\text{HbA}_{1\text{C}}$ ) test [21]. Serum fasting blood sugar (FBS) and glyated hemoglobin ( $\text{HbA}_{1\text{C}}$ ) levels were estimated spectrophotometrically using commercial kits (BIOLABO, France and Stanbio, USA, respectively) [22, 23]. An immunoenzymatic method was used to determine the serum level of insulin by using a specific kit (Rapid Insulin Test System, Product Code: 5825-

300, Monobind, USA) [24]. The Statistical Analysis System (SAS 2012) program was used to detect the potential effects of the investigated parameters in the different groups. The least significant difference –LSD test (Analysis of Variation-ANOVA) was used to compare between mean values of the studied parameters and test for significant differences [25].

## Results and Discussion

### Effects of zinc and vitamin C supplementation on levels of fasting blood sugar

Table 1 shows the levels of fasting blood sugar in the studied groups.

**Table 1-** Effect of different groups in FBS

Groups	Mean $\pm$ SE
	FBS (mg/dL)
<b>G: A:</b> Healthy control group	85.820 $\pm$ 1.106 d
<b>G: B:</b> Diabetic patients without foot ulcers, without supplements group	274.097 $\pm$ 13.639 a
<b>G: C:</b> Diabetic patients with foot ulcers without supplements group	287.709 $\pm$ 7.487 a
<b>G: D:</b> Diabetic patients without foot ulcers, with supplements group	198.644 $\pm$ 10.711 c
<b>G: E:</b> Diabetic patients with foot ulcers, with supplements group	230.188 $\pm$ 13.922 b
LSD value	28.874 **
Means having different letters in the same column differ significantly, ** (P $\leq$ 0.01).	

The results of the present study showed that the mean level of FBS in G: D (198.644  $\pm$  10.711 mg/dL) was highly significantly (P $\leq$ 0.01) lower compared with that in G: B (274.097  $\pm$  13.639 mg/dL). Also, FBS level in G: E (230.188  $\pm$  13.922 mg/dL) revealed highly significant (P $\leq$ 0.01) decline as compared with G: C (287.709  $\pm$  7.487 mg/dL). However, FBS levels in G: D (198.644  $\pm$  10.711 mg/dL) and G: E (230.188  $\pm$  13.922 mg/dL) indicated highly significant (P $\leq$ 0.01) differences in comparison to G: A (85.820  $\pm$  1.106 mg/dL).

The results of the present study agree with those of previous reports [17, 12, 26] that proved the role of zinc in glucose metabolism. Also, another study [10] demonstrated a potential role of vitamin C in decreasing blood glucose.

Zinc was reported to improve anti-diabetic activity [27]. It has an important role in the regulation of insulin production by the pancreas [28]. It rises the absorption of glucose by the hepatic, muscle, and fat cells [17]. Improving zinc levels can lead to improvements in glucose metabolism [28]. This indicates the ability of zinc in improving glucose metabolism, which results in reducing fasting blood sugar (FBS) levels. Vitamin C is known to be an antioxidant. The similarity in the structure between vitamin C and glucose makes it of importance in diabetes [29]. This explains the fact that vitamin C and glucose compete with each other [10]. The dehydroascorbic acid transporters are members of the GLUT family of facilitative glucose transporters, of which at least three isoforms, GLUT1, GLUT3 and GLUT4, are dehydroascorbic acid transporters [30], which indicates decreasing glucose levels after using vitamin C supplement.

### Effects of zinc and vitamin C on levels of glycated hemoglobin in the whole blood

Table 2 shows glycated hemoglobin levels in the studied groups.

**Table 2-** Effects of different groups in HbA<sub>1C</sub>

Groups	Mean $\pm$ SE
	HbA <sub>1C</sub> (%)
<b>G: A:</b> Healthy control group	5.753 $\pm$ 0.102 d
<b>G: B:</b> Diabetic patients without foot ulcers, without supplements group	10.306 $\pm$ 0.318 a
<b>G: C:</b> Diabetic patients with foot ulcers without supplements group	11.264 $\pm$ 0.485 a
<b>G: D:</b> Diabetic patients without foot ulcers, with supplements group	7.776 $\pm$ 0.316 c
<b>G: E:</b> Diabetic patients with foot ulcers, with supplements group	9.151 $\pm$ 0.459 b
LSD value	1.006 **
Means having with the different letters in same column differed significantly, ** (P $\leq$ 0.01).	

The results of the present study demonstrated that the mean level of HbA<sub>1C</sub> in patients of group G: D ( $7.776 \pm 0.316$  %) revealed a highly significant ( $P \leq 0.01$ ) decrease as compared with G: B ( $10.306 \pm 0.318$  %). Also, HbA<sub>1C</sub> level in G: E ( $9.151 \pm 0.459$  %) decreased significantly ( $P \leq 0.01$ ) in comparison with G: C ( $11.264 \pm 0.485$  %). On the other hand, the levels of HbA<sub>1C</sub> in G: D ( $7.776 \pm 0.316$  %) and G: E ( $9.151 \pm 0.459$  %) showed highly significant ( $P \leq 0.01$ ) differences in comparison to G: A ( $5.753 \pm 0.102$  %).

With respect to the HbA<sub>1C</sub> levels, the present study agreed with earlier studies [17, 26], that reported the role of zinc in decreasing HbA<sub>1C</sub> levels. Also a similar finding was stated by another work [10], which stated that vitamin C could contribute in decreasing blood glucose. A potential explanation to the slight decrease in HbA<sub>1C</sub> levels could be the short period for supplements given (only 4 weeks). This is a relatively short time in comparison to the age of human red blood cells (RBCs) which is 120 days. These cells are normally phagocytized by macrophages of splenic and hepatic sinusoids by the end of their life [31].

### Effects of zinc and vitamin C on levels of serum insulin

Table 3 shows insulin levels in the studied groups.

**Table 3-** Effect of different groups in Insulin hormone

Groups	Mean $\pm$ SE
	Insulin ( $\mu$ IU/ml)
<b>G: A:</b> Healthy control group	$6.248 \pm 0.240$ d
<b>G: B:</b> Diabetic patients without foot ulcers, without supplements group	$44.325 \pm 3.769$ b
<b>G: C:</b> Diabetic patients with foot ulcers without supplements group	$57.973 \pm 4.477$ a
<b>G: D:</b> Diabetic patients without foot ulcers, with supplements group	$33.826 \pm 4.621$ c
<b>G: E:</b> Diabetic patients with foot ulcers, with supplements group	$41.311 \pm 1.645$ bc
LSD value	10.177 **
Means having with the different letters in the same column differed significantly, ** ( $P \leq 0.01$ ).	

The results of the present study illustrated that levels of serum insulin in G: D ( $33.826 \pm 4.621$   $\mu$ IU/ml) revealed a highly significant ( $P \leq 0.01$ ) decline in comparison with G: B ( $44.325 \pm 3.769$   $\mu$ IU/ml). Levels of serum insulin hormone in G: E ( $41.311 \pm 1.645$   $\mu$ IU/ml) showed a highly significant ( $P \leq 0.01$ ) decrease in comparison with G: C ( $57.973 \pm 4.477$   $\mu$ IU/ml). However, G: D ( $33.826 \pm 4.621$   $\mu$ IU/ml) and G: E ( $41.311 \pm 1.645$   $\mu$ IU/ml) demonstrated highly significant ( $P \leq 0.01$ ) increases compared with G: A ( $6.248 \pm 0.240$   $\mu$ IU/ml).

The results of the present study agreed with those of earlier works [17, 26], which demonstrated a possible role for zinc in reducing insulin levels in the blood.

Zinc is involved in the synthesis, release, storage, secretion, and conformational integrity of insulin [28]. It increases sensitivity of insulin through increasing the binding capability to its receptors [32] by functioning as a cellular second messenger in the insulin signaling pathway and glucose homeostasis [33]. Notably, zinc is existent in the secretory vesicles within beta cells of the pancreas, where it contributes in insulin crystallisation/ storage and is therefore released together with insulin into the plasma [34]. In addition to the structural role of zinc in the stored form of insulin, it has the ability to modulate insulin action. Improving zinc levels in the body leads to improvement in insulin action [28]. While, vitamin C supplement assists in decreasing the oxidative stress in the skeletal muscles and enhances insulin sensitivity, which helps in glucose uptake [35].

On the other hand, zinc deficiency disrupts insulin hormone homeostasis [34]. Also, reduced levels of vitamin C in the plasma is stated in both insulin-dependent and non-insulin dependent diabetic patients [36]. This could explain the decreased insulin levels following the use of zinc and vitamin C supplements.



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